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ORIGINAL ARTICLE

A comprehensive evaluation of the crustacean assemblages in southern Iberian Mediterranean wetlands

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ABSTRACT

Although Mediterranean wetlands were recognized as biodiversity hotspots, most of them are nowadays threatened by human activities that have led to habitat loss and degradation. A total of 36 wetlands were monitored to assess species richness of branchiopods and copepods by using accumulation curves and non-parametric estimators. Three different types of wetlands were identified: i) temporary freshwaters-subsaline-hyposaline (TFSH); ii) permanent freshwaters-subsaline-hyposaline (PFSH); and iii) mesosaline-hypersaline (MH) wetlands (including temporary and permanent ones). A total of 60 species were recorded; they belong to seven different orders. A large number (37%) of rare species (present in only one wetland) were found while only 11% of the total species were common (i.e., present in more than 20% of wetlands). Species richness was related to wetland typology, with the largest number of species observed in TFSH, followed by MH and by PFSH wetlands. We have found that rare species are mainly present in temporary wetlands, the most vulnerable to hydrological changes; hence, these types of wetlands represent unique sites deserving conservation.

Key words: Biodiversity, conservation, Mediterranean wetlands, non-parametric estimators, zooplankton.

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INTRODUCTION

The Mediterranean region is considered as one of the most important biodiversity hotspots in the world (Myers *et al*., 2000). In particular, the southern Iberian Peninsula is an area of special interest as it is located in one of the most arid zones of Europe and comprises a wide range of aquatic ecosystems, from freshwater to hypersaline ones (Sánchez-Fernández *et al*., 2004), which represent an important component of the landscape.

Mediterranean wetlands are characterized by social, economic, cultural, scientific and environmental values (see Williams, 1999 among others). These ecosystems have been considered as unique because of their ecological characteristics, which frequently hold exclusive communities of aquatic organisms, and play an important role in the maintenance of regional biodiversity (Williams, 1999; De Meester *et al*., 2005; Oertli *et al*., 2005; Céréghino *et al*., 2008). However, this value has been frequently overlooked, contributing to their neglect and inadequate management (Semlitsch and Bodie, 2005). In fact, nowadays, Mediterranean wetlands are highly endangered, suffering widespread degradation and loss due to increase of intensive cultivation, livestock, and urban uses (Beja and Alcazar, 2003; García-Muñoz *et al*., 2010).

Zooplankton is one of the most important communities present in these wetlands. Zooplanktonic assemblages have previously been used for the ecological evaluation of wetlands (Gannon and Stemberger, 1978; Caramujo and Boavida, 2000; Bianchi *et al*., 2003; Boix *et al*., 2005; Parra *et al*., 2009). However, recently the European Water Framework Directive (WFD) has excluded this community from the evaluation criteria in the protection of wetlands. Some authors have considered this exclusion as an error (Jeppesen *et al*., 2011 and references therein), because the knowledge of zooplankton diversity is a necessary tool for the development of strategies for the management and protection of aquatic biodiversity at landscape level (Marrone *et al*., 2006; De Bie *et al*., 2008). Moreover, the lack of fish community in temporary wetlands, denote the importance to include zooplankton in the evaluation of ecological quality and conservation procedures in Mediterranean wetlands.

Previous studies focusing on zooplankton richness in the Mediterranean area are scarce in comparison to other climatic regions (Álvarez-Cobelas *et al*., 2005). The aim of this study has been the development of monitoring methods to evaluate zooplankton biodiversity (using as target groups copepods and branchiopods) in a Mediterranean area in southern Spain in order to build models for the entire Mediterranean region.

METHODS

Study wetlands

In the present study, we selected a total of 36 endorheic wetlands (Fig. 1) widely differing in their limnological characteristics (Tab. 1). The term endorheic reflects the closed nature (no outlet) of the drainage systems of wetlands (Dantín, 1929; 1940; Allan *et al.*, 1995). In particular the studied wetlands differ in altitude (265 to 1582 m asl) and in morphological characteristics: maximum area ranging from 0.2 to 46 ha and maximum depth from 10 to 456 cm (Ortega *et al*., 2003, 2006). All these wetlands are located in the Alto Guadalquivir, a representative region of the southern Iberian Peninsula (Spain), with a Mediterranean continental climate, a Mediterranean type of climate with continental tendencies (Capel Molina, 1981; Díaz de la Guardia *et al.*, 2003). The three main morphologic and geologic units that exist in Andalusia are present in the study area: Sierra Morena siliceous mountains at the north, the Guadalquivir depression with olitostromics deposits and Triassic rocks in the centre and Baetic calcareous mountains in the south and east (Vera, 1994). The valley of the Guadalquivir River represents the most important geographical entity of this district, being a large proportion of this area which is characterized by a semiarid climate with a mean annual precipitation around 400 mm, although aridity decreases from the Guadalquivir valley to mountain areas located in the north, south and east of this valley (AEMET, 2011).

Sampling

Accordingly to the high inter-annual variability that characterized Mediterranean wetlands, zooplankton samples (branchiopods and copepods) were collected seasonally during a multi-year sampling (1998-2002 and 2010, representing a great range of weather conditions that affect Mediterranean wetlands) to obtain cumulative zooplankton species richness (presence-absence data). In all cases, samples (integrated composite samples) were collected along two longitudinal transects, one from the

Fig. 1. Localization of the Alto Guadalquivir region (south of Spain) and wetlands included in the study.

shore line to the open water, and the other along the shore line. Sampling strategy allowed us to evaluate the crustacean assemblages considering the spatial and temporal heterogeneity characterizing the studied wetlands. Samples were collected through 63-µm mesh size plankton net and preserved *in situ* with 4% formalin (f.c.). Once in the laboratory, all organisms were identified to species level, according to Dussart (1967, 1969), Dussart and Defaye (1995), Einsle (1996), and Ranga-Reddy (1994) for copepods and Alonso (1996) for branchiopods.

Data analysis

Previous to analysis, wetlands were grouped based on the criteria obtained by Boix *et al.* (2005) for Mediterranean wetlands, according to conductivity and temporality values. In our case, the scarce number of saline wetlands in the study area (salinity threshold >5 g L^{-1}) prevented us from producing two categories in relation to temporality. Hence, three different categories of wetlands were identified: i) temporary freshwater-subsaline-hyposaline wetlands (TFSH); ii) permanent freshwater-subsaline-hyposaline wetlands (PFSH); and iii) mesosaline-hypersaline wetlands (MH).

In order to describe diversity patterns among different wetland groups, parameters such as singularity and species richness were estimated. For all parameters, presence-absence data of total zooplankton species (sum of branchiopods and copepods), total branchiopods and total copepods were considered. First, in order to identify those wetlands characterized by a greater number of unique species, a singularity index including the complete dataset, was applied (Boix *et al.*, 2008):

Tab. 1. Characteristics of the studied wetlands, ordered by increasing salinity from freshwater to hypersaline ones. Data obtained from this study and Ortega *et al*. (2003, 2006).

	Coordinates (UTM)	Altitude (m)	Maximum area (ha)	Maximum depth (cm)	Salinity (g L^{-1})
Casasola	30SUG8780	285	3.60	75	0.35
Perales	30SVH9548	757	5.20	105	0.38
Pedernoso	30SWH0047	724	1.40	110	0.45
Mojones	30SVG0877	493	4.50	122	0.51
Orcera	30SWH3542	1270	0.50	172	0.51
Santisteban	30SVH8134	637	3.00	90	0.51
Hituelo	30SVG0679	476	3.80	179	0.54
Fuensanta	30SVG5870	1000	1.30	22	0.56
Rumpisaco	30SVG1077	538	4.00	33	0.56
Siles	30SWH4249	1280	1.30	234	0.59
Nava de la Zarzuela	30SVG3219	1254	0.20	30	0.59
Nava del Hoyoncillo	30SVG2767	1465	0.28	30	0.59
Navas	30SVG0486	378	3.50	223	0.68
Naranjeros*	30SVG0978	508	5.20	456	0.76
Fernandina	30SVH4805	482	0.21	15	0.84
Grande*	30SVG5098	368	22.90	350	1.00
Ardal	30SVH4721	400	0.50	28	1.14
Tobaruela	30SVH4215	363	1.70	70	1.21
Cañada la Cruz	30SWH2713	1582	3.10	32	1.25
Chica	30SVG5098	370	5.80	80	1.25
Castillo	30SWH2357	780	0.60	147	1.33
Ranal	30SVG0697	340	10.70	81	1.42
Argamasilla	30SVG5392	484	4.80	220	1.50
Iruela	30SWG0322	1515	0.15	28	1.50
Villardompardo	30SVG1489	360	1.70	318	1.58
Huelma	30SVG5668	1224	2.00	10	1.66
Muela	30SWH0610	1324	5.40	35	1.76
Casillas	30SVG1084	442	2.70	241	2.15
Garcíez*	30SVG2389	441	7.90	355	3.10
Prados del Moral	30SVG2989	389	4.80	120	3.50
Chinche	30SUG9863	452	4.70	107	4.50
Conde	30SUG9359	412	46.00	120	24.82
Quinta	30SUG8679	289	7.70	199	24.82
Brujuelo	30SVG4191	458	4.20	212	33.19
Rincón del Muerto	30SUG8779	265	4.20	166	82.29
Honda*	30SUG9961	446	9.90	316	86.56

**Permanent wetland.*

$$S = \left({}^{e}\!/_{E}\right) \cdot 100 \qquad \text{(eq. 1)}$$

where S is the index of singularity for one wetland typology, e is the number of species found in one wetland typology and not found in other typology, and E is the total number of species found in this wetland typology.

In relation to the species richness, it is important to recognize the inherent difficulty for recording all species in a fauna inventory, especially for the case of invertebrates (Jiménez-Valverde and Hortal, 2003; Hortal *et al.*, 2006). As a result, different methods were proposed in the literature for estimating the number of species in a given area (González-Oreja *et al.*, 2010). In this study, two approaches were used to provide valid comparisons among the inventories of species of the different wetland groups, and so to evaluate the completeness of crustacean species inventories. Estimations were performed by randomised resampling with replacement from a pooled group of samples; this implies that each species can appear in any pooled sample. Walther and Moore (2005) suggested that the generated data are independent of one another. Samples-based curves were obtained using EstimateS 8.2.0 software. The first approach was based on species accumulation curves, in which cumulative species richness is plotted against the number of samples (Moreno and Halffter, 2000). Species accumulation curves were obtained by using EstimateS 8.2.0 software. Subsequently, Clench equation (*Sn*) was assigned to describe the species accumulation model, allowing us to obtain an ideal function that describes the species accumulation with the increase of sampling effort (Soberon and Llorente, 1993; Moreno and Halffter, 2000; Hortal *et al.*, 2006):

$$S_n = a \cdot n/(1 + b \cdot n) \qquad \text{(eq. 2)}$$

where a is the rate of increase of new species at the beginning of the inventory, b is a parameter related to the shape of the curve, and n is the sample effort. Data were adjusted by using non-linear estimation of Hooke-Jeeves, using Statistica 7.0 software. This fitting is based on geometric procedures to minimize the loss function (see Jiménez-Valverde and Hortal, 2003; StatSoft, 2004). The parameters of the Clench equation gave us information about: i) the reliability of our inventories (*Ri*); ii) the rate of recorded fauna (*Rf*); iii) the sampling effort needed to identify all species (*nq*); and iv) the total number of theoretical species present in each wetland type (*Nts*):

$$R_i = a/(1 + b \cdot n)^2 \qquad \text{(eq. 3)}$$

$$R_f = S_{obs}/(a/b) \qquad \text{(eq. 4)}$$

S_{obs}: observed species richness

$$n_q = q/[b \cdot (1 - q)] \qquad \text{(eq. 5)}$$

q: percentage of recorded species (in our case 90%)

$$N_{ts} = a/b \qquad \text{(eq. 6)}$$

In our study we have considered that *Ri* values lower than 0.1 indicated that our inventories were near to be complete (Hortal and Lobo, 2002). In other studies, the inventories were considered as representative when 70% of estimated identified species were collected (Hortal and Lobo, 2002).

In the second approach, rarefaction curves (Longino *et al.*, 2002) were used to obtain an estimation of total zooplankton richness for each wetland group. In particular, the observed richness (*Sobs* - Mao Tau), and two non-parametric estimators, based on presence-absence data sets, were calculated: i) ICE (Incidence-based Coverage Estimator); and ii) corrected version of Chao2 (Incidence-based estimator). The corrected Chao2 version was used because some problems were detected when the infrequent species were unique (Colwell, 2005). Both estimators were calculated with 100 randomizations (Colwell, 2005; Hortal *et al.*, 2006) and by using the software EstimateS 8.2.0. Finally, in order to quantify the performance of the above mentioned non-parametric estimators, we have calculated bias, precision and accuracy for *Sobs* (Mao Tau), ICE and corrected Chao2 [see Walther and Moore (2005) for more detailed information]. In brief, bias (PAR) reflects under- or overestimates of the true value of species richness. Precision (CV) is measured as the coefficient of variation, without considering if the estimator is biased or not. Accuracy (SRMSE) takes into account the difference between the estimated and the total species. The ideal characteristics for a good estimator are bias values close to zero and small precision values (Walther and Martin, 2001).

$$PAR = \frac{1}{n}\sum_{j=1}^{n}\left(100E_j/A\right) \qquad \text{(eq. 7)}$$

$$CV = \frac{100SD}{\bar{E}} \qquad \text{(eq. 8)}$$

$$SRMSE = \frac{1}{A}\sqrt{\frac{1}{n}\sum_{j=1}^{n}\left(E_j - A\right)^2} \qquad \text{(eq. 9)}$$

where A is the asymptotic or total species richness (calculated following Colwell, 2005); E_j is the estimated species richness for the j_{th} sample; and n is the number of samples.

RESULTS

Crustacean composition

A total of 60 species (branchiopods and copepods) were recorded in the Alto Guadalquivir region, belonging to seven orders (Tab. 2). Twenty-two of the recorded species were copepods (7 Calanoida, 12 Cyclopoida and 3 Harpacticoida), and 38 were branchiopods (4 Anostraca, 1 Ctenopoda, 32 Anomopoda and 1 Notostraca). A large number (37%) of rare species (present in only one wetland) were found (15 branchiopods and 7 copepods

Tab. 2. Zooplankton species richness in the Alto Guadalquivir region.

	Casasola	Perales	Pedernoso	Mojones	Orcera	Santisteban	Hituelo	Fuensanta	Rumpisaco	Siles	Nava de la Zarzuela	Nava del Hoyoncillo	Navas	Naranjeros	Fernandina	Grande	Ardal	Tobaruela	Cañada la Cruz	Chica	Castillo	Ranal	Argamasilla	Iruela	Villardompardo	Huelma	Muela	Casillas	Garcíez	Prados del Moral	Chinche	Conde	Quinta	Brujuelo	Rincón del Muerto	Honda
ANOMOPODA																																				
Alona azorica (Frenzel & Alonso, 1988)									X								X												X							
Alona elegans (Kurz, 1875)													X																							
Alona iberica (Alonso & Pretus, 1989)		X																																		
Alona quadrangularis (Müller, 1776)							X							X		X																				
Alona rectangula (G. O. Sars, 1862)						X															X		X		X											
Alona salina (Alonso, 1995)																																	X			
Bosmina longirostris (Müller, 1776)							X									X		X		X			X										X			
Cerioaphnia pulchella (G. O. Sars, 1862)										X																										
Ceriodaphnia dubia (Richard, 1894)			X		X																				X											
Ceriodaphnia laticaudata (P. E. Müller,1867)		X																X			X	X						X								
Ceriodaphnia quadrangula (Müller, 1785)			X		X	X	X		X				X				X				X									X			X			
Ceriodaphnia reticulata (Jurine, 1820)									X														X											X		
Chydorus sphaericus (Müller, 1776)					X																															
Daphnia (Daphnia) curvirostris (Eylmann, 1887)																					X															
Daphnia (Ctenodaphnia) hispanica (Glagolev & Alonso, 1990)		X				X															X															
Daphnia (Ctenodaphnia) magna (Strauss, 1820)							X							X		X		X		X		X	X		X				X		X		X			
Daphnia (Ctenodaphnia) mediterranea (Alonso, 1985)																																X			X	X
Daphnia (Daphnia) parvula (Fordyce, 1901)																		X																		
Dunhevedia crassa (King, 1853)		X					X																X							X						
Estateroporus gauthieri (Alonso, 1990)										X											X															
Leydigia leydigii (Schödler, 1863)																X																				
Leydigia acanthocercoides (Fischer, 1854)																					X															
Macrothrix hirsuticornis (Norman & Brady, 1867)							X				X		X		X								X					X			X		X			
Macrothrix laticornis (Jurine, 1820)		X																			X															
Moina brachiata (Jurine, 1820)						X	X		X			X			X		X																			
Moina micrura (Kurz, 1875)	X	X				X										X					X															
Moina salina (Daday, 1888)																																X			X	
Pleuroxus aduncus (Jurine, 1820)																																	X			
Pleuroxus letourneuxi (Richard, 1888)			X			X																									X		X			X
Simocephalus exspinosus (DeGreer, 1778)							X						X										X										X			
Simocephalus vetulus (Müller, 1776)		X	X		X	X				X							X				X															
Tretocephala ambigua (Lilljeborg, 1900)																	X																			
ANOSTRACA																																				
Artemia sp. (Leach, 1819)																																			X	
Branchipus schaefferi (Fischer, 1834)																	X																			
Chirocephalus diaphanus (Prévost, 1803)			X	X		X				X					X		X		X		X			X		X		X		X						
Tanymastix stagnalis (Linnaeus, 1758)		X																																		

To be continued on next page

Tab. 2. Continued from previous page.

	Casasola	Perales	Pedernoso	Mojones	Orcera	Santisteban	Hituelo	Fuensanta	Rumpisaco	Siles	Nava de la Zarzuela	Nava del Hoyoncillo	Navas	Naranjeros	Fernandina	Grande	Ardal	Tobaruela	Cañada la Cruz	Chica	Castillo	Ranal	Argamasilla	Iruela	Villardompardo	Huelma	Muela	Casillas	Garcíez	Prados del Moral	Chinche	Conde	Quinta	Brujuelo	Rincón del Muerto	Honda
CTENOPODA																																				
Sida crystallina (Müller, 1776)							X																													
NOTOSTRACA																																				
Triops cancriformis (Lamarck, 1801)																	X																			
CALANOIDA																																				
Arctodiaptomus salinus (Daday, 1885)																																X	X	X	X	X
Arctodiaptomus wierzejskii (Richard, 1888)							X													X		X	X													
Copidodiaptomus numidicus (Gurney, 1909)																X																				
Diaptomus cyaneus (Gurney, 1909)			X		X																X															
Hemidiaptomus robaui (Richard, 1888)		X																																		
Mixodiaptomus incrassatus (Sars, 1903)		X			X																															
Neolovenula alluaudi (Guerne & Richard, 1890)							X		X	X				X							X				X			X								
CYCLOPOIDA																																				
Acanthocyclops sp.							X																					X								
Acanthocyclops vernalis (Fischer, 1853)					X									X		X													X	X						
Cyclops abyssorum (Sars, 1863)							X																													
Cyclops sp. 1																																	X			
Cyclops sp. 2		X	X			X				X																										
Cyclops strenuus (Fischer, 1851)									X								X						X													
Diacyclops bicuspidatus (Claus, 1857)																									X											
Macrocyclops albidus (Jurine, 1820)										X																										
Megacyclops viridis (Jurine, 1820)																			X						X		X				X					
Metacyclops minutus (Calus, 1863)	X	X	X	X		X		X	X		X		X		X	X	X			X	X	X				X		X		X				X		X
Metacyclops planus (Gurney, 1909)							X					X		X																						
Microcyclops rubellus (Lillejeborg, 1901)						X	X																													
HARPACTICOIDA																																				
Canthocamptus microstaphylinus (Wolf, 1905)																					X															
Canthocamptus staphylinus (Jurine, 1820)		X	X		X																															
Cletocamptus retrogressus (Schmankevitch, 1875)						X							X	X						X						X						X	X	X	X	X

species; Tab. 2), while only 11% of the total species number (4 branchiopods and 3 copepods species) were common (present in more than 20% of the wetlands).

Biodiversity

Species richness differed between the three wetland typologies previously mentioned, with the largest number of species recorded in TFSH (51 species; 4±2 species per sample), followed by MH (16 species; 3±1 species per sample) and PFSH (12 species; 3±1 species per sample). Fig. 2 shows that the highest values of singularity for branchiopods, copepods and total zooplankton species were also found in TFSH wetlands. The accumulation curves obtained by using Clench equation in the different wetland types (Tab. 3), have reflected that inventories were near to be completed and could be considered as representative, with values of reliability below 0.1. The rate of recorded fauna was around 85% evidencing that sampling strategy was effective, except for the case of branchiopods in MH wetlands, with a 73% rate of recorded fauna. In general, results for the three wetland types have shown that the species accumulation curves obtained by using both, observed richness (Mao-Tau) and non-parametric estimators (ICE and Chao-2) were close to reaching the asymptote (Fig. 3). This result again confirms that a significant proportion of species richness has been effectively surveyed, and they also agree with the values of reliability, close to 0.1 (Tab. 3).

Finally, in relation to the quality of the non-parametric estimators, ICE was the best estimator for predicting species richness as it was reflected by values which were less biased, more precise and accurate (Tab. 4).

DISCUSSION

This study has revealed that Alto Guadalquivir wetlands are generally characterized by rich crustacean

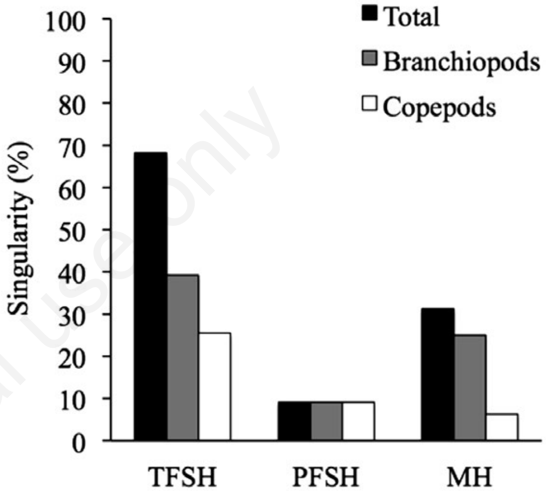

Fig. 2. Values of singularity obtained for each wetland type.

Tab. 3. Results of species accumulation curves (Clench equation) for the different wetland types.

	Total	Branchiopods	Copepods
TFSH			
Cumulative species richness	51	32	19
R2 Clench model	0.9997	0.9998	0.9992
Reliability of inventory	0.0794	0.0507	0.0285
Rate of registered fauna (%)	85.51	84.88	86.61
Sampling effort necessary to determine 90% of species	135	140	126
Total number of theoretical species	54	34	20
PFSH			
Cumulative species richness	12	6	6
R2 Clench model	0.9973	0.9992	0.9917
Reliability of inventory	0.0926	0.0451	0.0478
Rate of registered fauna (%)	83.42	83.61	83.02
Sampling effort necessary to determine 90% of species	38	36	41
Total number of theoretical species	13	7	6
MH			
Cumulative species richness	16	12	4
R2 Clench model	0.9990	0.9990	0.995
Reliability of inventory	0.0657	0.0831	0.0040
Rate of registered fauna (%)	86.38	72.56	97.56
Sampling effort necessary to determine 90% of species	59	120	11
Total number of theoretical species	16	13	4

TFSH, temporary freshwaters-subsaline-hyposaline wetlands; PFSH, permanent freshwaters-subsaline-hyposaline wetlands; MH, mesosaline-hypersaline wetlands.

assemblages, similarly to other areas of the Iberian Peninsula (Boronat *et al*., 2001; Frisch *et al*., 2006; Fahd *et al*., 2009; Gascón *et al*., 2009; Caramujo and Boavida, 2010), and to other Mediterranean wetlands (Ripley and Simovich, 2009; Alfonso and Belmonte, 2011). There are several reasons for explaining why the study area is an excellent site for analysing the coupling between zooplankton species richness and composition, with some forcing environmental factors. First, the high zooplankton species richness of the study site, with the presence of several species with special relevance to the Spanish and Andalusia context such as, *Copidodiaptomus numidicus*, a typical species of the North African, Sardinia and southern Italy fauna, and *Neolovenula alluaudi*, a species of tropical and Ethiopic origin (Miracle, 1982; Alfonso and Belmonte, 2013); and some species endemic to the Iberian Peninsula such as *Alona iberica*, *Alona salina*, and *Daphnia hispanica* (Alonso, 1996). Second, the existence, in this area, of a wide variety of wetland types in a broad range of ecological gradients (especially in terms of temporality and salinity, see Ortega *et al*., 2003; Guerrero *et al*., 2006; Gilbert *et al*., 2014).

Salinity and hydroperiod are the two main drivers taken into account when classifying Mediterranean wetlands (Boix *et al*., 2005). Previous studies have observed that salinity and temporality are key factors for determining crustacean species richness and composition in Mediterranean wetlands (Alonso, 1998; Marrone *et al*., 2006; Brucet *et al*., 2009). In relation to salinity, we have found typical species of FSH wetlands in terms of occurrence (*i.e. Chirocephalus diaphanus* and *Moina branchiata*), and others that appears only in MH ones (*i.e*., *Arctodiaptomus salinus*, *Daphnia mediterranea* and *Moina salina*; Tab. 2). However, certain species such as *Cletocamptus retrogressus* or *Metacyclops minutus*, with wide ranges of salinity (Alonso, 1998; Boronat *et al*., 2001), have masked this pattern, because these species appear in a broad range of wetlands.

Concerning hydroperiod, the scarcity of studied permanent wetlands, an inherent difficulty of the Mediterranean climate (Guerrero, 2009) reduces the probability of finding typical species. Moreover, most of the species found in permanent wetlands were also present in temporary ones. Nevertheless we have detected some differences in the occurrence of zooplankton species between TFSH (*i.e*., *Moina brachiata*, *C. diaphanus*, *Cyclops* sp. 2 and *Simocephalus vetulus*) and PFSH (*i.e*., *C. numidicus* and *Leydigia leidigii*). In saline waters, the lack of a clear distinction as a function of hydrology contrasts with other climatic regions for which there is substantial evidence indicating differences in faunal composition related to hydrology, hence reinforcing the idea of the different structure and functioning of Mediterranean wetlands (Eitam *et al*., 2004; Álvarez-Cobelas *et al*., 2005).

Our results also suggest that increasing overall salinity has a negative effect on total species richness, since 85% of the species occur in FSH *vs* 26% in MH. In fact, it is well-known that high salinity values increases biota stress, reducing growth and reproduction rates (Sarma *et al*., 2006) and leaving the community with only salinity-tolerant species adapted to these particular habitat types (Por, 1980). In relation to composition, the total ratio copepod: total zooplankton species richness was lower in saline than in freshwater wetlands, probably due to the dependency of copepods on less fluctuating environmental conditions (Brucet *et al*., 2005, 2006). By contrast, the total ratio branchiopods: total zooplankton

Tab. 4. Performance of non-parametric estimators for the Alto Guadalquivir data set.

	Total			Branchiopods			Copepods		
	Mao-Tau	ICE	Chao2	Mao-Tau	ICE	Chao2	Mao-Tau	ICE	Chao2
TFSH									
Predicted species richness	48	50	50	30	31	30	18	20	19
Bias	68.62	85.04	85.04	67.67	82.01	75.41	70.23	89.73	83.57
Precision	27.53	11.69	11.69	27.75	15.10	17.65	27.16	14.59	20.39
Accuracy	0.37	0.18	0.18	0.37	0.22	0.28	0.35	0.17	0.24
PFSH									
Predicted species richness	11	13	12	6	6	6	5	7	6
Bias	70.56	99.41	81.67	68.42	87.08	71.27	72.91	110.96	82.59
Precision	24.73	18.67	23.98	25.85	21.78	23.77	23.51	21.98	25.57
Accuracy	0.34	0.18	0.26	0.36	0.23	0.33	0.32	0.26	0.27
MH									
Predicted species richness	16	16	15	12	13	12	4	4	4
Bias	70.42	83.26	71.56	58.51	74.22	58.72	87.95	93.17	84.96
Precision	26.08	17.05	22.29	31.65	26.85	29.10	13.30	10.28	10.99
Accuracy	0.22	0.22	0.32	0.45	0.32	0.45	0.17	0.12	0.18

TFSH, temporary freshwaters-subsaline-hyposaline wetlands; PFSH, permanent freshwaters-subsaline-hyposaline wetlands; MH, mesosaline-hypersaline wetlands.

species richness was greater in saline wetlands, reflecting the higher salinity tolerance of those species in Mediterranean region (Brucet *et al*., 2009).

The natural fluctuations characterizing Mediterranean wetlands imply a restriction on the assessment of the species richness in a short-temporal scale. Therefore, the cumulative richness in a long time-period provides a larger sample size and so offers a better knowledge of the species richness composition (Fahd *et al*., 2009). Moreover, the application of these models is recommended when comparing species diversity from sites that differ in their characteristics (Moreno and Halffter, 2000), and the probability of finding new species is improved by increasing the sampling effort (Soberón and Llorente, 1993). Our results for nine species accumulation curves (considering a six-year sampling period), fitted to Clench model, have shown values of recorded fauna of approximately 85%, except in the case of branchiopods richness in saline waters, hence confirming that zooplankton species diversity in the study area has been properly estimated. The cumulative species richness in relation to the total number of theoretical species has differed slightly, except for branchiopods in saline wetlands. Furthermore, the sampling effort necessary to achieve this percentage is arduous, representing a higher effort-cost relationship. Our results suggest that, similar to previous studies (Vandekerkhove *et al*., 2005), the sampling effort must be more intense in temporary environments with high temporal dynamics and species turnover rates. By contrast, efficient sampling effort is likely to be lower in permanent environments where species replacement occurs slowly. In addition, Shurin *et al*. (2007) noted that the determination of species richness estimated in short time periods was a good indicator of the biodiversity. In our case, the typical characteristics of Mediterranean wetlands, where the species replacement occurs more quickly, justifies the need for longer time-scale sampling (Fahd *et al*., 2009). Despite the advantages offered by the accumulation curves to determine the number of species, several studies have demonstrated the suitability of rarefaction curves for reinforcing these results (Hortal *et al*., 2006). Three aspects must be taken into account when applying this methodology (Walther and Martin, 2001). Firstly, an error measure associated to an estimator is not very useful. Secondly, it is necessary to establish an appropriate sampling unit, and thirdly, the performance of the estimators depends on the quality of the data. As a result, and considering that our sampling effort was relatively low (six-years but only seasonal sampling), the use of two non-parametric estimators (ICE and Chao2) which are unbiased and precisely accurate (Hortal *et al*., 2006) justified this approach in fluctuating environments. However, in all cases the most appropriate estimator (ICE) tended to underestimate the species richness, except for the case of copepods in PFSH wetlands.

Species richness and singularity were much higher in temporal than in permanent freshwater wetlands. Similar results, related to species richness, were documented in other Mediterranean wetlands (Frisch *et al*., 2006; Boix *et al*., 2008). One likely explanation is the well-known intermediate-disturbance hypothesis (Connell, 1978) where recurrent disturbances (alternation of flood and droughts) favour the removal of dominant species making the resources available to a larger number of species (Denslow, 1985). That is, the number of species that can coexist increases directly with increasing environmental variance. In this replacement dynamic, the existence of an egg bank (De Stasio, 1989; Vandekerkhove *et al*., 2005; Angeler, 2007; Esteban and Finlay, 2010; Galotti *et al*., 2014) could play an important role in the presence of one or another species. The zooplankton dormant stages are considered one of the most important elements in the colonisation process during early temporary pond succession (Angeler *et al*., 2008). So these cryptic stages are an essential part of the zooplankton community for the maintenance of biodiversity at regional scale in a context of global change. Furthermore, another plausible explanation is the presence of vagrants or tourist species, *i.e*., rare species which only occasionally appear and do not belong to the community. These vagrants could increase artificially the species richness in temporary wetlands repeatedly sampled. Nonetheless, they may be important elements in the biodiversity of these ecosystems because play a significant role on the ecosystem resilience (Jiménez-Valverde and Hortal, 2003).

CONCLUSIONS

Hydrology and salinity are crucial factors determining species richness and composition of zooplankton (branchiopods and copepods) in Mediterranean wetlands. We have found that the most vulnerable wetlands, the temporary ones, are unique sites for supporting rare aquatic species not present in permanent wetlands. As Collinson *et al*. (1995) indicated, the conservation of temporary wetlands should seek to protect all taxa, and micro-crustaceans should be included in future conservative legislation. Moreover, it is important to note that Mediterranean region is considered as a sensitive area to climatic changes (Sánchez-Fernández *et al*., 2004). Although Mediterranean water bodies are characterized by extreme natural water level fluctuations in response to irregular precipitation patterns (Álvarez-Cobelas *et al*., 2005), global climatic change is projected to amplify this pattern as a consequence of: i) the increase of temperatures; ii) the elevation of evapotranspiration rates; iii) the diminution of precipitation levels; and iv) the increase of summer droughts (Coops *et al*., 2003; Angeler,

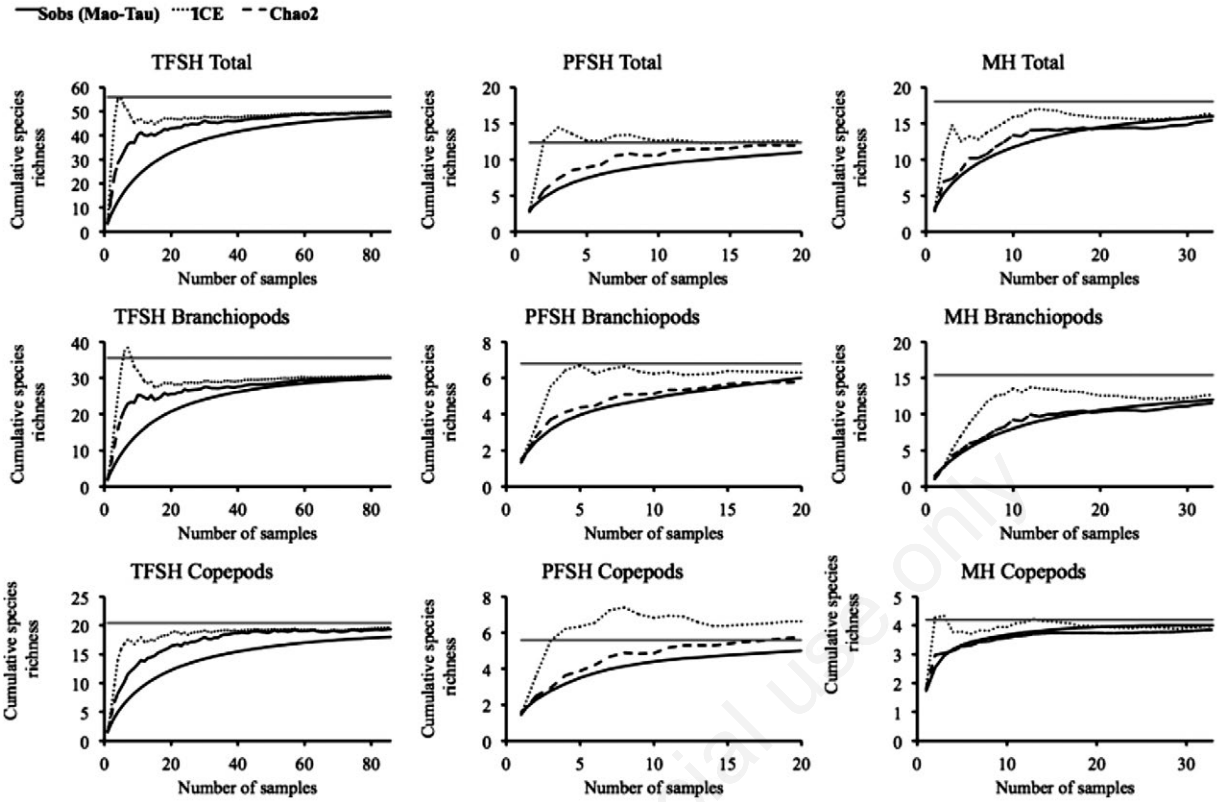

Fig. 3. Zooplankton species richness observed (*Sobs*) and non-parametric estimators (ICE and Chao2) used to predict the species richness in different wetland types. Each graph shows the total value of species richness (grey solid line), according to Colwell (2005).

2007; García-Jurado *et al*., 2012). These fluctuations will be most pronounced in closed-basin lakes, where the balance between precipitation and evaporation controls water levels (Marsh and Lesack, 1996). As a direct result of all these processes, changes in hydrology (*i.e*., flooding period and water permanence) and salinity are expected (Beklioglu *et al*., 2007). All these changes would affect the species that inhabit them, because their ability to adapt the new environmental conditions is limited, which would increase extinction rates and the loss of biodiversity (Hughes, 2000; McCarty, 2001; Walther *et al*., 2002; Root *et al*., 2003). The adequate knowledge about species composition and diversity that support these systems would increase our ability to manage them and to implement efficient conservation strategies that could help mitigating future effects of global climate change (Angeler, 2007). Even more, Mediterranean wetlands are also seriously affected by human impacts (agriculture or urban development) that induce other forms of stress that will make more unpredictable and complex the biological responses to climate change (IPCC, 2007).

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